

Science Supporting Online Material (34 pages)

**Accumulation of Mn(II) in *Deinococcus radiodurans*
Facilitates Gamma-Radiation Resistance**

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Materials and Methods

Strains. Unless otherwise indicated, *Deinococcus radiodurans* (USUHS) (ATCC BAA-816); *Deinococcus grandis* (DSM 3963); *Deinococcus geothermalis* (DSM 11300); *Shewanella oneidensis* (MR-1) (ATCC 700550); *Pseudomonas putida* (ATCC 47054); *Enterococcus faecium* (ATCC 19434); *Lactobacillus plantarum* (ATCC 14917); and *Escherichia coli* (strain K-12) (MG1655) (provided by Dr. M. Cashel, NIH). Superoxide dismutase mutant *sodA*⁻ *D. radiodurans* (KK7004) (provided by Dr. K. K. Wong, Baylor College of Medicine, Houston, TX, USA).

Growth. Cells were typically inoculated into liquid medium at $\sim 1 \times 10^6$ CFUs/ml followed by incubation at 32°C. For each trial in liquid media, or CFU assays on solid medium for IR- or desiccation-survival, three replicates were carried out; standard deviations are shown in the figures. Undefined rich media (TGY) (1% bactotryptone, 0.5% yeast extract, and 0.1% glucose). Liquid defined minimal medium (DMM) was prepared as described in Table 1(I) of Venkateswaran *et al.* (2000), but supplemented with cysteine (50 µg/ml), histidine and methionine (each at 25 µg/ml), and Mn(II) (manganous chloride) was added as the only transition metal cation, unless otherwise stated; no Fe was added to DMM. The effects of Fe(II) (ferrous sulfate), Co(II)

(cobalt chloride), Mo(II) (ammonium molybdate) or [Cd(II) (cadmium sulphate) (2.5 μ M) + Mn(II) (2.5 μ M)] were tested in DMM (Fig. 3B, main text). Defined rich medium (DRM) (i.e., DMM which is highly enriched with Cys, His, Met, Glu, Ala, Arg, Gly, Lys, Pro, Ser, Thr, Val and Leu [cell culture quality]) was prepared as described in Table 1(II) of Venkateswaran *et al.* (2000), but with amino acids added at 250 μ g/ml each and supplemented with or without Mn(II) (no-Mn DRM); no Fe or other transition metal cations were added to DRM. When investigating the effect of Mn(II) on solid medium, DMM (Fig. 4A, main text), DRM (Fig. 4B, main text; and fig. S3) or TGY (fig. S4B) plates were solidified with Noble agar (DIFCO) pre-treated with multiple 1 mM EDTA washes, followed by EDTA removal, which eliminates contaminating Mn(II), but not Mn(III) or (IV). For Fe-chelator studies (Fig. 3C, main text), cells were pre-grown in DMM to OD₆₀₀ 0.9, irradiated (⁶⁰Co, 9 kGy) and inoculated (1/20 dilution) into DMM +/- 50 μ M Dp (2,2'-dipyridyl) and 50 μ M Ds (deferoxamine mesylate). In all cases, non-irradiated control cells were pre-grown and inoculated as for irradiated cells. Late-log phase (LLP) for *Deinococcus* in TGY corresponds to OD₆₀₀ 0.9 after incubation for ~12 h at 32°C; early-stationary phase (ESP) for *Deinococcus* in TGY corresponds to OD₆₀₀ 1.2 after incubation for ~24 h at 32°C; in DMM, ESP for *Deinococcus* corresponds to OD₆₀₀ 0.9 after ~48 h.

Transmission Electron Microscopy (TEM). Bacterial suspensions were rinsed in 0.1 M cacodylate buffer (pH 7.4), fixed in 2.5% gluteraldehyde in the same buffer, and post-fixed in 1% osmium tetroxide. Fixed samples were embedded in Epon-Araldite resin, and 50-70 nm sections were stained with uranyl acetate followed by lead citrate. Samples were examined with a Philips CM 100 electron microscope. Additional TEM images supporting Fig. 1 (main text) can be found at http://www.usuhs.mil/pat/deinococcus/index_20.htm.

⁵⁹Fe and ⁵⁴Mn accumulation. Cells were cultured aerobically as follows: *D. radiodurans* and *D. geothermalis* in DMM were grown to OD₆₀₀ 0.9, and *S.*

oneidensis in defined *Shewanella* medium (DSM) (to OD₆₀₀ 0.9) consisting of 3 mM 4-piperazinediethanesulfonic acid (PIPES, pH 7.0), 30 mM NaCl, 28 mM NH₄Cl, 4.4 mM KH₂PO₄, 1.3 mM KCl, 20 mM Na lactate and 10 mL/L each of trace mineral and vitamin solutions. The cells in each culture were harvested by centrifugation, washed twice in an equal volume of phosphate-buffered saline (PBS, pH 7.0) with a final wash in PBS/1 mM EDTA, and suspended in DMM or DSM, respectively. For each of the cultures, radiolabeled metal was added to cell suspensions of 6.3 × 10⁷ and 1 × 10⁹ CFU/mL for *D. radiodurans* and *S. oneidensis*, respectively, at a final concentration of 0.018 and 0.0057 μCi/ml, respectively, for ⁵⁹Fe or ⁵⁴Mn (Isotope Products Laboratories; ⁵⁹Fe 100 Ci/g; ⁵⁴Mn 10 Ci/g). For determination of ⁵⁹Fe and ⁵⁴Mn uptake, the labelled cells were harvested by centrifugation after incubation for 24 h at 30°C. Cells were filtered onto 0.2 μm nitrate cellular filters and rinsed with 6 mL of cold (4°C) medium. Filters were suspended in 15 ml of liquid scintillation fluid (Optifluor, Packard Bioscience) and counted with a liquid scintillation counter (model 1411, Wallac). Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (100 μM) (SI) was added and cells were incubated at 30°C for 1 h before adding either ⁵⁹Fe or ⁵⁴Mn. In assessing the number of atoms of ⁵⁹Fe or ⁵⁴Mn accumulated per cell, cell density was determined by direct microscopic counting after staining with acridine orange to allow resolution of individual cells, whether they occurred singly, in pairs, or in tetrads. For *D. radiodurans*, 1.08 × 10⁵ ⁵⁴Mn atoms/cell corresponds to ~2.74 mM, assuming an average cell volume of 6.5 × 10⁻² μm³ (from Fig. 1E and 3D, main text).

Acute ⁶⁰Co Irradiations. The indicated strains (Fig. 2, main text; and fig. S3A) were inoculated in liquid TGY, DMM or DRM at ~1 × 10⁶ CFUs/ml and grown to OD₆₀₀ ~0.9 in the presence or absence of Fe chelators, 50 μM 2,2'-dipyridyl (Dp) and 50 μM deferoxamine mesylate (Ds). Cells were then irradiated without change of broth on ice with ⁶⁰Co at 8 kGy/hour (⁶⁰Co Gammacell irradiation unit [J. L. Shepard and Associates, Model 109]). At the indicated doses, cultures were appropriately diluted

and plated on solid medium, and CFU counts were determined after 5-7 days' incubation at 32°C. For Fig. 4B (main text), *D. radiodurans* was pre-grown to OD₆₀₀ 0.8 in defined rich medium (DRM) without Mn(II) supplementation (no-Mn DRM) and irradiated to 9 kGy or 12 kGy without change of broth. A fixed number of control and irradiated cells ($\sim 4 \times 10^5$ CFUs) were transferred to the indicated segments, and plates were incubated at 32°C for 7 days.

Chronic ¹³⁷Cs Irradiations. Chronic irradiation was delivered in a ¹³⁷Cs Gammacell 40 irradiation unit [Atomic Energy of Canada Limited]), typically for 5-7 days at 32°C. For Fig. 4A (main text), cells were pre-grown to OD₆₀₀ 0.9 in DMM supplemented with 50 nM Mn(II), 100 nM Mn(II) or 250 nM Mn(II). A fixed number of cells ($\sim 1 \times 10^6$ CFUs) were transferred to the indicated segments. For Fig. 4C (main text), cells were pre-grown in TGY to OD₆₀₀ 0.9, with plate inoculations and incubations as for Fig. 4A (main text). Under 50 Gy/hour, plates were covered but not sealed during incubation to allow O₂ diffusion.

Supporting Online Material (SOM) Text

Comparative genomics of radiation resistant bacteria

Manganese-dependent microorganisms such as *Deinococcus*, *Arthrobacter*, *Bacillus*, *Streptococcus* and cyanobacteria spp. have been implicated in the deposition of manganese oxide in dark manganiferous rock varnish coatings on desert rocks (S2, S3). Organisms that belong to those groups are known for their radiation and desiccation resistance, and the current work establishes a link between the role of Mn(II) and an environmental niche they occupy. At least five bacteria reported to be highly radiation resistant (*D. radiodurans*, *D. geothermalis*, *L. plantarum*, *Rubrobacter xylanophilus*, and *Kineococcus radiotolerans*) have been subjected to genome sequencing (S4), and

comparative analyses support that their DNA repair systems are encoded by a variety of common genes (tables S1 and S2). However, no shared group of uncharacterized genes has been identified in those sequenced organisms that might comprise an expanded gene core involved in recovery. Viewed in this context, it is possible that *D. radiodurans* and other radioresistant bacteria use relatively conventional DNA repair and protection systems, but with greater efficiency than other organisms. Our results for *D. radiodurans* (main text) indicate that accumulation of high intracellular Mn together with relatively low Fe concentrations is an important factor in radiation resistance. Conversely, cells reported here with high Fe and low Mn contents are relatively radiation sensitive (Table 1, main text).

Differences between CFU-survival and cell-survival were only significant for *D. radiodurans* grown in TGY or DRM

The slightly greater resistance of *D. radiodurans* cells grown in TGY compared to DMM at doses >10 kGy (Fig. 2, main text) has been reported previously (S5) and is believed to be the result of cell-grouping. Whereas four cells of a tetracoccus need to be killed to eliminate a colony-forming unit (CFU) only two cells of a diplococcus need to be killed; this yields radiation survival curves with pronounced shoulders for cultures comprised mostly of tetracocci. Therefore, in assessing the radiation resistance of bacterial cultures, failure to correct for cell-grouping can lead to exaggerated resistance values based on CFU counts.

For the survival curve of *D. radiodurans* (TGY, open triangle) presented in Fig. 2 (main text), the cell-grouping was ~75% diplococci and ~25% tetracocci (Fig. 1A, main text). Under the assumptions that the survival of cells, constituting the CFU,

is independent from each other, that survival of a single cell is enough to ensure the survival of a CFU, and that the relative frequency of a k -cell CFU is known to be f_k ($\sum f_k = 1$), the relationship between the individual cell-survival and the CFU-survival follows the equation: $p(x) = \sum f_k p_k(x) = \sum f_k (1 - [1 - p_1(x)]^k)$ where, for a radiation dose x , $p(x)$ is the survival probability for an arbitrary CFU in the mixture, $p_k(x)$ is the survival probability for a k -cell CFU and $p_1(x)$ is the survival probability for an individual cell. For a culture consisting of diplococci and tetracocci (3:1), survival of 17% of individual cells (D_{17}) translates to 37% of CFU-survival (D_{37}); i.e., for the TGY culture (Fig. 2, open triangle, main text), the D_{17} of individual cells is ~ 12 kGy. For affect of cell-grouping in DRM (16 cells/CFU), see fig. S3. Cell-grouping for *E. coli* and *S. oneidensis*, and the other organisms, was typically 1 cell/CFU, ie., the differences between CFU-survival and cell-survival were only significant for *D. radiodurans* grown in TGY or DRM (Fig. 1A and 2, main text; and fig. S3).

Relationship between ionizing radiation and DSB damage

Several studies have shown that for a given dose of radiation delivered under anaerobic conditions, the number of DSBs in *D. radiodurans* compared to other bacteria is about the same (~ 0.004 DSB/Gy/Genome), and that its DNA is not endowed with unusual protection from *in vivo* irradiation (S6). Consistently, we have shown that for a given dose of γ -radiation delivered under aerobic conditions the numbers of irradiation-induced DSBs/haploid genome induced in *D. radiodurans*, *E. coli* and *S. oneidensis* are approximately 0.01 DSB/Gy/Genome (fig. S2). This value corresponds to the 0.0114 DSBs/Gy/genome (200 DSBs/genome at 17.5 kGy)

previously determined by TEM of DNA prepared from agarose-embedded *D. radiodurans* cells grown and irradiated aerobically (S7).

Irradiation- and metabolism-induced sources of ROS (Reactive Oxygen Species)

Cell-survival depends not only on the amount of damage accumulated during irradiation, but also on the levels of ROS (oxidative stress) produced by metabolism during recovery, which is dependent on cell density, O₂ levels and the composition of recovery substrate (S8, S9). Alone or in combination, both sources of ROS can lead to cell death by damaging DNA, RNA, proteins and lipids (S8). In contrast to HO·, which are highly damaging to all biomolecules (S8, S10), O₂·⁻ and H₂O₂ are substantially less oxidizing and considered to contribute only moderately to oxygen enhancement of radiation-induced DNA damage; consistently, only 2.5-fold fewer DSB/Gy occur in *D. radiodurans* anaerobically (S6) versus in air (S7) (fig. S2). Since high intracellular Mn(II) concentrations are known to efficiently scavenge O₂·⁻ (S10), and Mn(II) does not participate in Fenton-type chemistry (S10), it is possible that cellular components such as DNA or enzymes highly susceptible to ROS (S8) are less likely to encounter O₂·⁻, and H₂O₂-derived HO· in *D. radiodurans* during recovery than organisms that accumulate relatively low Mn levels (Table 1, main text). Metabolism-induced toxicity has been reported for many bacteria, causing mutations and inhibiting energy metabolism; tricarboxylic acid (TCA) cycle activity is strongly suppressed by O₂·⁻ (S8). For example, growth of *E. coli* double mutants (*sodA*⁻*B*⁻) lacking Mn-SOD and Fe-SOD is inhibited in minimal medium in air because of elevated oxidative stress (S8). We previously reported that irradiated (15 kGy) *D.*

radiodurans strongly represses the O_2^- radical-generating step (*sdhB*) (*S11*) during recovery in TGY, but at the same time, the glyoxylate bypass of the TCA cycle was induced, which could provide biosynthetic intermediates for recovery without generating high levels of ROS (*S11*).

In experiments on *E. coli* where the synthesis of SOD was externally controlled, it was shown that cells have calibrated their defenses so that they barely withstand the toxic action of normal levels of endogenous O_2^- (*S8*). *E. coli*, *S. oneidensis* and *P. putida* encode similar sets of SOD, catalase and peroxidase genes (table S3), and have low intracellular Mn and high Fe levels (Table 1, main text.). Therefore, recovery of irradiated cells dependent on enzymic ROS-protection systems might be compromised by their inability to recalibrate defenses in time to counter sudden increases in Fe-dependent endogenous oxidative stress generated during recovery (*S12*). This could lead to Fe(II)-induced error-prone DNA repair, mutagenesis and low survival levels (*S13*).

Growth of *D. radiodurans* in Fe-limited media

We tested *D. radiodurans* in DMM that contained the Fe chelators 2,2'-dipyridyl (Dp, Fe(II)-chelator) and deferoxamine mesylate (Ds, Fe(III)-chelator). *D. radiodurans'* growth and resistance were similar in DMM in the absence or presence of Dp and Ds (Fig. 2 and Fig. 3C, main text). Aerobic growth of bacteria in Fe-limited medium containing Fe chelators is unusual (*S1*). For example, the Gram-negative, dissimilatory metal-reducing bacterium *S. oneidensis* is an organism that accumulates Fe (Fig. 3D and Table 1, main text), contains an abundance of heme-containing c-type cytochromes (*S14*) and cannot grow in the presence of Dp + Ds (Fig. 4C, main text).

Other bacteria previously reported to accumulate high intracellular Mn and low Fe levels include *L. plantarum* and *Borrelia burgdorferi* (S1, S10). *Lactobacilli* are facultative anaerobes and frequently isolated as surviving contaminants from irradiated (5 kGy) meat (S15), and the spirochete *B. burgdorferi* is an extracellular pathogen adapted to host iron limitation (S1). Assimilation of Mn(II) by *L. plantarum* and *B. burgdorferi* appears to depend on the presence of a transmembrane proton gradient since ⁵⁴Mn uptake in these bacteria is blocked by CCCP (S1). Bacteria that have an absolute requirement for Mn(II) have been reported to be able to grow in Fe-limited media (S10), and *Deinococcus* (Fig. 3C, main text), *Lactobacillus* and *Borrelia* spp. fit this paradigm (S1). *D. radiodurans*, *L. plantarum* and *B. burgdorferi* have similar capacities to accumulate Mn atoms ($\sim 10^5\text{-}10^6$) on a per cell basis, and very low intracellular Fe levels have been reported in *L. plantarum* and *B. burgdorferi* (S1, S10).

We determined how extracellular Fe levels affect Fe accumulation in *D. radiodurans*. Cells cultured in DMM (2.5 μM Mn(II)) prepared with 2 mM phosphate buffer (instead of 20 mM) (S5) and buffered with 10 mM Tris (pH 8.0) grew (slowly) to OD₆₀₀ 0.8 in 3 days. Those cells were examined by ICP-MS and contained 4.72 (± 0.092) nmol Mn/mg protein, and undetectable levels of Fe. Liquid DMM prepared with 20 mM phosphate buffer contains 1.8 μM Fe; Tris-DMM, ~0.2 μM Fe. In contrast, *E. coli* ceases to grow when extracellular Fe concentrations drop below 0.4 μM (S16).

Mn(II) transport and regulation systems

Although the role of accumulated, non-enzymic Mn(II) in protection against oxidative stress in the radiation resistant lactobacilli (*S10, S15*) has been established for over two decades, the possibility that other radioresistant bacteria might use such a defense strategy has received almost no consideration. The precise mechanism by which Mn(II) scavenges O₂^{•-} is not understood (*S10*).

Mn(II) assimilation strategies that support the recovery of extremely resistant organisms such as *Deinococcus* spp. might also depend on expression of high catalase activities (*S17*), suppression of HO[•] production by limiting cellular Fe requirements (Table 1, main text) and restricting O₂^{•-} production by metabolic regulation (*S11*).

Similar arguments can be made to explain how other radiation resistant vegetative cells or bacterial spores that accumulate Mn(II) (*S10*) can also survive desiccation (*S18*); the desiccation resistance profiles of the six representative bacteria reported here (fig. S5) mirror the trends in their intracellular Mn levels and radiation resistance profiles (Table 1, Fig. 2 and Fig. 4C, main text; and fig. S3).

At least three types of Mn(II) import systems have been described since 1995. The unusual transporter from *L. plantarum* is a P-type ATPase, the only one to date that has been demonstrated to have high specificity for Mn(II) (*S19*). The other two types of Mn(II) uptake system conform to either the ATP-binding cassette (ABC) transporter superfamily or to the natural resistance-associated macrophage protein (Nramp) family. For *D. radiodurans*, we have identified two predicted Mn transporters. First, DR1709 belongs to the Nramp family of transporters (COG1914) (fig. S6A). On the COG phylogenetic tree it occupies one branch with the experimentally characterized *E. coli* and *S. typhimurium* Mn-transporters MntH (*S20*).

The second predicted Mn transporter is an ATP-dependent ABC-type transporter (DR2283-DR2284, DR2523) (fig. S6B-D). This type of transporter has been experimentally characterized for a few bacteria (S21-S26), including *Synechocystis* (S21, S22) and *Bacillus subtilis* (S23). The ABC-type Mn transporter typically is organized as a single operon encoding three proteins: periplasmic binding protein (fig. S6B), ATP-ase (fig. S6C) and a permease (fig. S6D) (some bacteria may have an additional permease (fig. S6D)). For *D. radiodurans*, the most probable ABC-type Mn transport candidates are: DR2283 (permease, COG1108), DR2284 (ATP-ase, COG1121) and DR2523 (periplasmic/surface adhesion, COG0803) (fig. S6).

Mn(II)-sensing occurs widely in bacteria and influences both Mn(II) homeostasis and genes involved in the oxidative stress response (S10). DR2539 (“long form” variant) (fig. S7A and B) of *D. radiodurans* belongs to the MntR/DtxR family of transcriptional regulators and its metal-binding motif is more closely related to the Mn(II)-binding configuration than the Fe(II) form (fig. S7A). On the COG’s phylogenetic tree, DR2539 is located on the same branch as TroR, which is a “short form” repressor from *Treponema pallidum* (fig. S7A) (S27).

Cadmium-induced radiation sensitivity

Competitive inhibition of Mn(II)-dependent transport by Cd(II) has been observed for a variety of Mn(II)-accumulating bacteria (S10). Consistently, we found that growth rates in DMM for *D. radiodurans* are reduced by Cd(II); 2.5 μ M Cd(II) inhibited growth in high-Mn DMM (Fig. 3B, main text) and 50 nM Cd(II) in low-Mn DMM. The effect of 1.0 μ M Cd(II) in TGY plates was similar to Mn(II)-restriction in low-Mn DMM, both conditions inhibited growth of the wild-type under chronic radiation

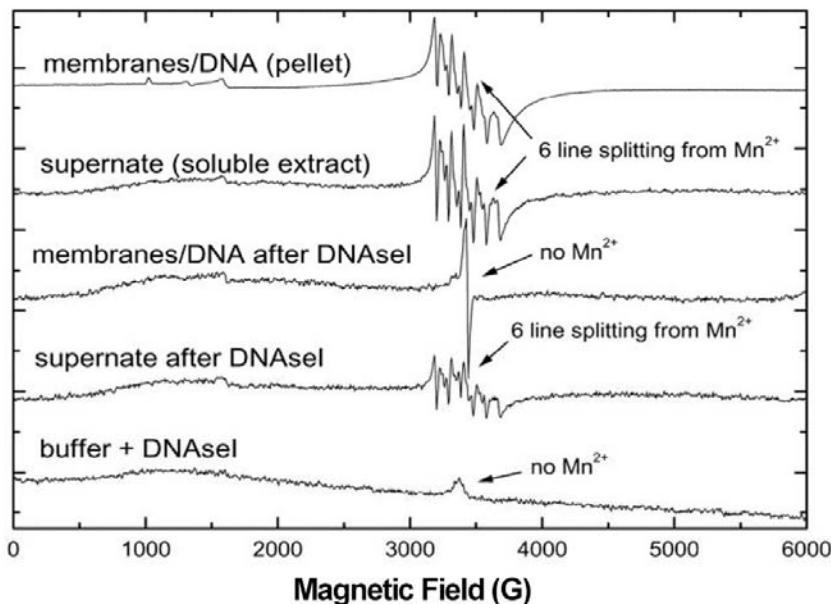
but not in the absence of irradiation (Fig. 4A, main text; and fig. S4A). We also tested the effect of Cd(II) on acutely irradiated cells recovering on TGY. Growth of cells on TGY medium was not affected by 1.0 μ M Cd(II), but Cd(II) delayed recovery of acutely irradiated cells (fig. S4B). We found that the *D. radiodurans* Mn-SOD (*sodA*⁻) mutant KKW7004 (S28) could grow under chronic radiation on TGY, but not in the presence of Cd(II) (fig. S4A). Thus, Cd(II) might contribute to toxicity under 50 Gy/hour (fig. S4A) or following acute irradiation (fig. S4B) by competitively inhibiting Mn(II) transport, and hence increasing oxidative stress.

Relevance of results to other work

Mn-based SOD-mimetic compounds (S29-S32) and Fe-chelators (S12, S33) have been shown to be effective in promoting radiorecovery in animals. Reelfs *et al.*, (2004) (S12) have reported the immediate release of labile “free” Fe in skin cells as a consequence of oxidative damage during irradiation. Consistent with our hypothesis that scavenging of ROS after irradiation is critical to recovery (main text), the origin of the radioresistance of the human osteosarcoma cell line HS-Os-1 has also been attributed to the strong scavenging of the cells for ROS following irradiation (S34).

Supporting Online Figures S1 to S7

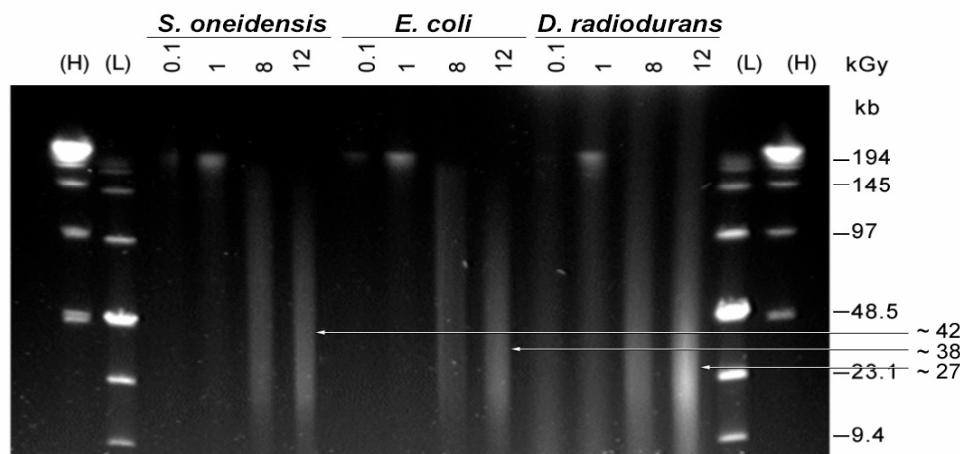
Daly_Fig. S1



S1. Legend. Electronic Paramagnetic Resonance (EPR) spectroscopy of *D. radiodurans* (ATCC BAA-816). Cells were grown in DMM (main text) to the early-stationary phase (OD_{600} 0.9), subjected to French Pressure cell disruption, and followed by ultracentrifugation. The soluble extract and pellet fractions were treated by DNaseI and then EPR samples were frozen by slow immersion in liquid nitrogen. X-band EPR spectra were acquired using a Bruker E500 spectrometer (Billerica, MA) equipped with an Oxford Instruments ESR-10 liquid helium cryostat, with the following conditions: 2 K; microwave power, 801 μ W; modulation amplitude, 10 G; microwave frequency, 9.614260 GHz. Mn(II) typically has an intense signal at $g = 2.00$, due to the $M_s = -1/2$ to $+1/2$ transition, with 6-fold splitting from hyperfine interaction with the $I = 5/2$ manganese nucleus (S35). *Micrococcus luteus* (Sarcina lutea) and *E. coli* have very low paramagnetic Mn(II) compared to *D. radiodurans*.

(S35). *M. luteus* is Gram-positive and is similar in size, shape and cell wall composition to deinococci, staphylococci, streptococci and enterococci, but sensitive to ionizing radiation, ultraviolet light and desiccation (S36, S37).

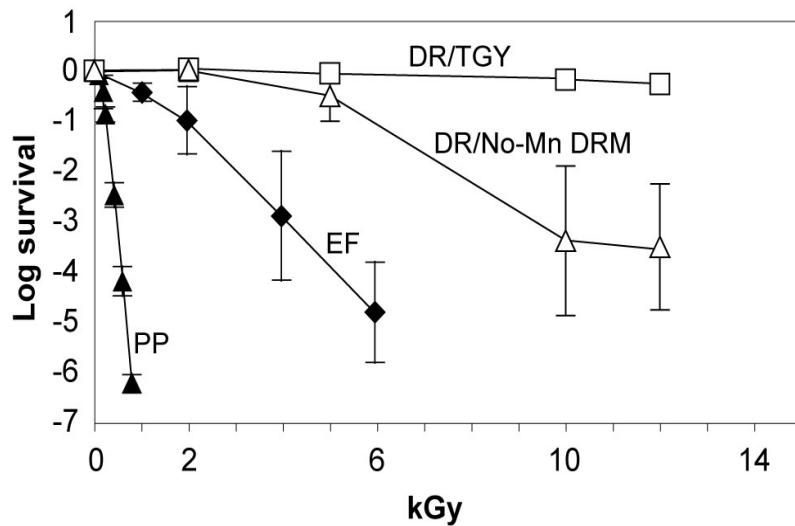
Daly_Fig. S2



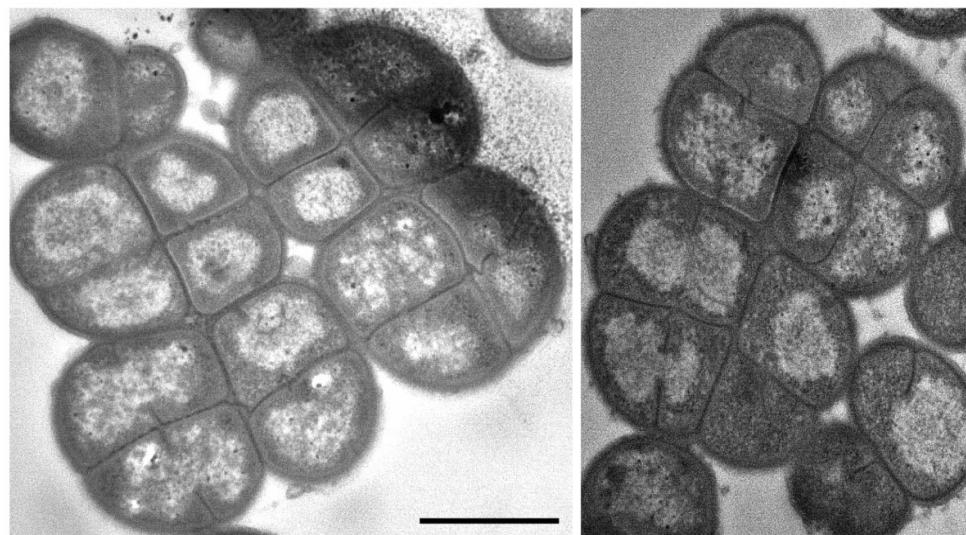
S2, Legend. Pulse Field Gel Electrophoresis (PFGE) of genomic DNA prepared from irradiated *S. oneidensis* (5.1 Mbp), *E. coli* (4.6 Mbp) and *D. radiodurans* (3.3 Mbp). Cultures were grown aerobically to the late-log phase (OD_{600} 0.9) in TGY medium at 32°C, and γ -irradiated aerobically on ice without change of broth with ^{60}Co to 0.1, 1.0, 8.0, and 12 kGy. A fixed number of control and irradiated cells ($\sim 1 \times 10^6$ CFUs) were embedded in agarose, lysed, and subjected to PFGE as described previously (S38, S39). The DNA was visualized by ethidium bromide staining. The markers in the first two and last two lanes are PFGE standards (low (L) and high (H) range PFGE markers) from New England Biolabs, with sizes shown on the right (kbp). The approximate average fragment sizes for 12 kGy samples are indicated on the far right,

and correspond to ~0.01 DSB/Gy/Genome. This PFGE data is consistent with other reports that *D. radiodurans* DNA is not endowed with unusual protection from *in vivo* irradiation (*S6, S39*).

Daly_Fig. S3A



Daly_Fig. S3B

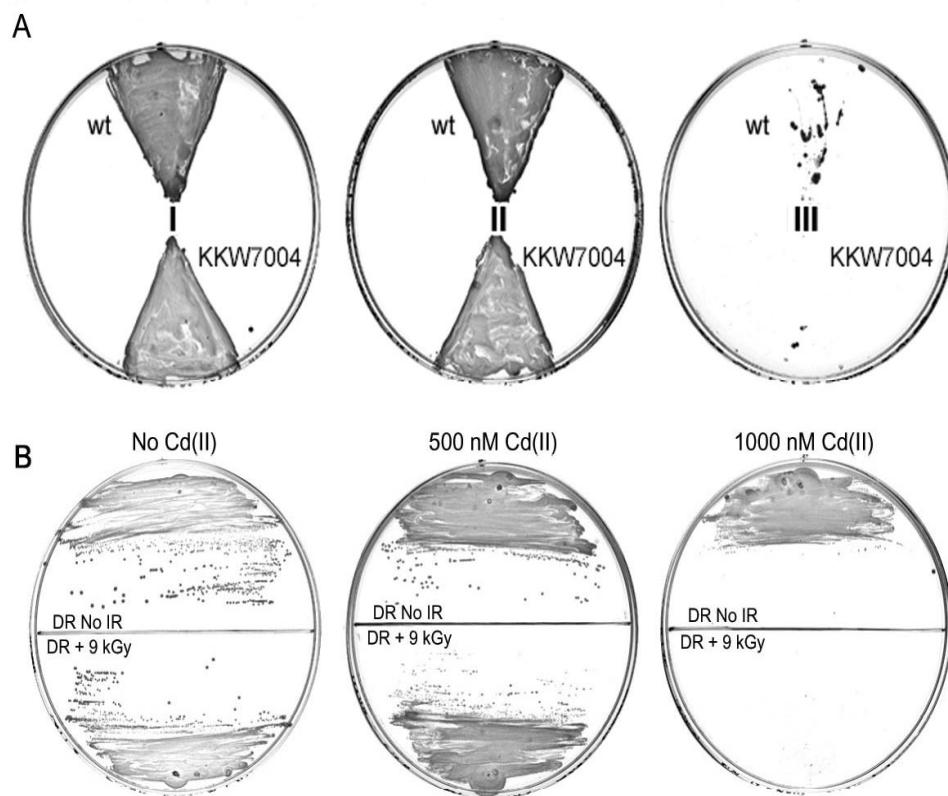


S3, Legend. (A) Survival of strains exposed to acute doses of γ -radiation. Solid triangle, *Pseudomonas putida* (PP) (ATCC 47054), pre-grown in TGY (OD₆₀₀ 0.9),

recovered on TGY (D_{10} , 0.25 kGy). Solid diamond, *Enterococcus faecium* (EF) (ATCC 19434), pre-grown in TGY (OD_{600} 0.9), recovered on TGY (D_{10} , 2 kGy). Open triangle, *D. radiodurans* (DR) (ATCC BAA-816), pre-grown in DRM without Mn(II) supplementation (no-Mn DRM, Materials and Methods) (OD_{600} 1.0), recovered on no-Mn DRM (DR/No-Mn DRM). Open square, *D. radiodurans* (DR), pre-grown in no-Mn DRM (OD_{600} 1.0, same cultures as for ‘open triangle’), recovered on TGY (DR/TGY). At the indicated doses, cultures were appropriately diluted and plated on solid medium, and CFU counts were determined after 7 days’ incubation at 32°C. Values are from three independent trials, with standard deviations shown. Non-irradiated DR cultures grown in no-Mn DRM to OD_{600} 1.0 (3 days’ growth) gave rise to essentially the same number of CFUs/ml when plated on either no-Mn DRM or TGY ($\sim 2 \times 10^7$ CFUs/ml). At OD_{600} 1.0, cells grown in no-Mn DRM typically clustered as ≥ 4 tetracocci/CFU (i.e., 16 cells/CFU) (panel B), yielding an adjusted D_{10} cell-survival value of ≤ 2.5 kGy (i.e., for a culture comprised of 16 cells/CFU, 81% CFU-survival translates to 10% cell-survival) (SOM Text). The D_{10} cell-survival value of ~ 2.5 kGy for the wild-type is quantitatively similar to the resistance reported for several IR-sensitive *D. radiodurans* DNA repair mutants (S48-S50). At 10 kGy, *D. radiodurans* grown in no-Mn DRM (OD_{600} , 1.0) displays a 1,000-fold reduction in CFU-survival on no-Mn DRM compared to TGY. *D. radiodurans* grown in no-Mn DRM contained 0.11 (± 0.01) nmol Mn/mg protein and 2.65 (± 0.05) nmol Fe/mg protein (Mn/Fe ratio, 0.04) (ICP-AES (Atomic Emission Spectrometry)). (B) Two TEM images of *D. radiodurans* grown in no-Mn DRM to OD_{600} 1.0, showing cell-grouping. Scale bar 1 μ m.

The survival curve for *Deinococcus geothermalis* (D₁₀, 10 kGy) and growth under chronic radiation was previously reported by Brim *et al.* (2003) (S40). For growth of bacteria on TGY plates under chronic radiation, *E. faecium* displayed luxuriant growth under 50 Gy/hour in the presence or absence of the Fe-chelators Dp and Ds (each, 125 μ M); *P. putida* did not grow under 50 Gy/hour with or without Fe-chelators; and *D. radiodurans* did not grow on no-Mn DRM under 50 Gy/hour.

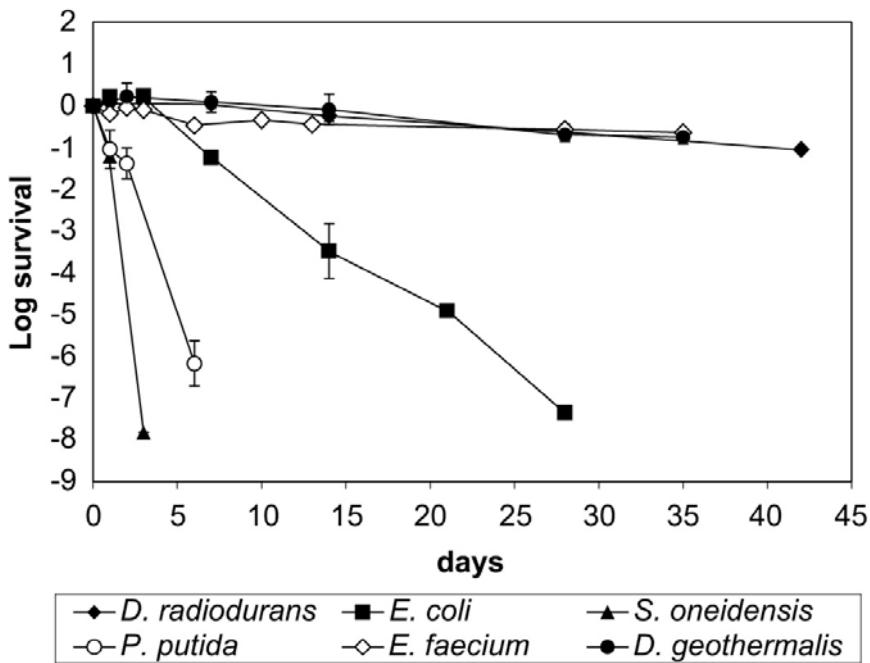
Daly_Fig. S4



S4, Legend. Cd(II) inhibits recovery from γ -radiation. **(A)** Effect of Cd(II) on the growth of *D. radiodurans* wild-type (wt) (ATCC 13939) and *sodA*⁻ mutant (KKW7004) (S28) on TGY under chronic radiation. A fixed number of cells ($\sim 1 \times 10^6$ CFUs) were transferred to the indicated segments, and plates incubated for 5 days at

30°C in a ^{137}Cs irradiator (main text). I, TGY (Km 10 $\mu\text{g/ml}$) + 50 Gy/hour; II, TGY (Km 10 $\mu\text{g/ml}$) + 1.0 μM Cd(II), no radiation; III, TGY (Km 10 $\mu\text{g/ml}$) + 1.0 μM Cd(II) + 50 Gy/hour. Control, wild-type (wt) *D. radiodurans* (ATCC 13939 (parent of KKW7004) + pMD66 (an autonomously replicating plasmid encoding Km resistance) (*S39*)). KKW7004 and wt/pMD66 were pre-grown in TGY/Km 25 $\mu\text{g/ml}$ to OD₆₀₀ 0.9. **(B)** Cd(II) delays recovery of *D. radiodurans* exposed to acute γ -radiation (IR). *D. radiodurans* (DR) (ATCC BAA-816) was pre-grown in TGY to OD₆₀₀ 0.9, irradiated on ice to 9 kGy without change of broth, and recovered on TGY containing the indicated amount of Cd(II). A fixed number of control and irradiated cells ($\sim 1 \times 10^6$ CFUs) were transferred to the indicated segments, and plates were photographed after incubation at 32°C for 3 days. After 5 days' incubation, a bacterial lawn of *D. radiodurans* became visible on the 1000 nM Cd(II) plate on the segment labeled 'DR + 9kGy.' For *D. radiodurans*, Cd(II) is not bactericidal, but substantially reduces growth rates above 2.5 μM in high-Mn DMM (2.5 μM Mn(II)), and 50 nM in low-Mn DMM (25 nM Mn(II)). At 1 μM Cd(II), growth rates in liquid TGY are not significantly affected and cultures reached a final OD₆₀₀ of 1.2 in 24 hours.

Daly_Fig. S5



S5, Legend. Desiccation resistance of *S. oneidensis*, *P. putida*, *E. coli*, *E. faecium*, *D. geothermalis* and *D. radiodurans*. Cells were grown in TGY to OD₆₀₀ 0.9 ($\sim 1 \times 10^8$ CFUs/ml) and viable cell counts (by CFU assay) were determined prior to the desiccation experiments by plating appropriate dilutions on TGY. For desiccation resistance assays, samples (each containing $\sim 1 \times 10^6$ CFUs in TGY) were transferred to a microtiter plate, which was placed in a desiccation chamber over anhydrous calcium sulfate (WA Hammond Drierite CO. LTD., OH). The desiccation chamber was hermetically sealed and stored at room temperature. At the indicated times, cells were re-suspended in TGY, and CFU-survival was determined by dilution plating on TGY. Standard deviations shown.

Daly_Fig. S6 (A) Alignment for Nramp Mn-transporter (DR1709).

DR1709	VRRI-LPFLGPAVIASIAYMDPGNFATNIEGGARYGYSSLWVILAANLMAMVIQNLANSNLGIASGRNLPELIRERWPR
STM2408	ARKLRLALMGPAPIAAIGYIDPGNFATNIQAGASFYQLLWWVVVWANLMAMLIQILSAKLGIAATGKNLAEQIRDHYPR
EC_mntH	ARKMRLALMGPAPIAAIGYIDPGNFATNIQAGASFYQLLWWVVVWANLMAMLIQILSAKLGIAATGKNLAEQIRDHYPR
BS_ydaR	FRGL-LPFLGPAFIAAIAYIDPGNFATNISAGSKYGYMLLWVILFSNIMALLIQILSAKLGIAATGKNLPEVAREEFPK
CAP0063	LKGL-LKFLGPAFVVSVAYIDPGNFATNISGGSSFNYNLIWVILWSNLMALIQTMSAKLGIAATGCSLPECAKVFHK
MT0951	LKTS-WYLLGPAFVAAIAYIDPGNFATNISGGSSFNYNLIWVILWSNLMALIQTMSAKLGIAATGCSLPECAKVFHK
all17601	WRKM-LAYAGPGYLVSVGYIDPGNWATDIAGGSKFGYTLLTVILLNSNLMAILLQLSLCVRLGVATGRDLAQACRDYFSP
mlr2501	FRRL-FAFMGPYGMVSVGYMDPGNWATDLAGGAQFGYTLLFVIMLSNLMAILLQALAARLGIAATGRDLAQACRAYYPR
DR1709	PLVWFYWIQAEELVAMATDIAEFLGAALAIQLLTGLPMFWGAVVTGVVTFWLNLQ-KRGTRPLELAVGAFVLMIGVAY
STM2408	PVVFYIWVQAEELIAMATDIAEFIGAAIGFKLILGVSSLQGAVLTGIAATFLILMLQ-RRGQKPLEKVIGGLLFVAAAY
EC_mntH	PVVFYIWVQAEELIAMATDIAEFIGAAIGFKLILGVSSLQGAVLTGIAATFLILMLQ-RRGQKPLEKVIGGLLFVAAAY
BS_ydaR	PVSIGLWIQGELELIAATDIAEFIGAAALGGLLFLIPMLEASIAIAIGSFAILELQ-RRGYSLEAGIAGMLFVVVIAF
CAP0063	RANWIFWIVGELGAMATDIAEFIGGTGLGLLFLIPMIYAGLTLGVLTFIIVYME-KYGGKMETIIAAALIAVICVAY
MT0951	PARLAYWAQAEIVAMATDVAEVIGGAAIALRIMFNLPLPIGGIITGVVSLLLTIQDRRGQRLFERVITALLLVIAIGF
all17601	KVSFLCWLCETIAAACDIAELLSGATALQLLFVPLIWCVCITAIDVLVLLFLQ-HKGFRYTEALVIMLVATVGICF
mlr2501	PVNFWLWIACELIAIACDIAEVIGTAIAKLLFGIPLIGGAILTALDAFLVLLLM-NKGFRYLEAFVIALLIIIFSCF
DR1709	LVQVVLARPDLAAVGAGF-VPR--LQPGPSAYLAVGIIIGATVMPHVIYLHSALTQGRQI-TDTTEEKRRLVRLNRVD
STM2408	IVELEFFSQPDMAQLGKGMVIPA--LPNPEAVFLAAGVLGATIMPHVIYLHSSLTQHLH-GGTRQQR--YSATKWD
EC_mntH	IVELEIFSQPNLAQLGKGMVIPS--LPTSEAVFLAAGVLGATIMPHVIYLHSSLTQHLH-GGSRQQR--YSATKWD
BS_ydaR	ALQTFPAKPDAVSMVKGLFVPA--FHGTDVSLAAGILGATVMPHAIYLHSALTQRRVV-GKTDAERKIKIFRFEFID
CAP0063	TIELFLARPAWTQVMGHTLIPS--LPNGEAVLIAVGMILGATVMPHVIYLHSELVQHRNT-NSSDKEKLHLHKMKEID
MT0951	TASFFVVTPPPNAVLGG--APR--FQCTESVLLAAAAMGATVMPHHAVALHSGLARDRGRHDPGPQRRLRVTRWD
all17601	TAEIILFSRPDMGGILLG-YLPKKEILQNPEMLYIAIGILGATVMPHNLYLHSSIVQTRDW-QPTTEKRWEAIKFGTID
mlr2501	AIQIFVAAPPAGTILHSMFVPSSEIVTNPAMLYIAIGILGATVMPHNLYLHSSIVQTRAY-ERTEKGKRDAIKWATT
DR1709	VIAAMGLAGLINMSMLAVAATAFHGKNVENAGDLTTAYQTLTPLGP-AASVLFAVALLASGLSSSAVGTIMAGDVIMQ
STM2408	VAIAMTIAGFVNLMAMATAAAAFHFSGHTGIAIDLQAYLTLEPLLSH-AAATVFGSLVLAAGLSSTVVGTLAGQVVMQ
EC_mntH	VAIAMTIAGFVNLMAMATAAAAFHFSGHTGVALDDEAYLTLPQLLSH-AAATVFGSLVLAAGLSSTVVGTLAGQVVMQ
BS_ydaR	ILIAIMIAGAINASMLIVAAALFKKNG-LFVEDLDVAFQQFGHVLSP-MSAALFGIGLLVAGLSSSSVGTLSGDVIMQ
CAP0063	ILIAIMIAGFVVAAMIVVSAAFVKHG-IKVSTIEEAHRSILQPLLN-LSGGAFGIALASGLSSSAVGTIMAGQTIMK
MT0951	VGLAMIAGGVNAAMLLVAALNMR--GRGDTASIEGAYHAVHTDLGA-TIAVLFAVGLLASGLASSSSVGAYAGAMIMQ
all17601	STFALSLALFINSAILIVSAATFHFSGNQNVAEQDAYKLLSPLLGVSAASAIIFGIALLASGSSTLTATLAGQIVME
mlr2501	STIALMLALFVNAAILIVSAVAFHNTGHQDVAEIDQAFELSPLLGLCIASILFAVALLASGLNSTVTATLAGQIME
DR1709	GFMGFHIPLWLRRLIT---MLPAFIVI-LLGMDPS-SVLILSQVILCFGVPFALVPLLIEFARRDVVMGALVTRRSFT
STM2408	GFVFRHIPLWVRRIT---MLPSFIVI-LMGLDPT-RILVMSQVLLSFGIALALVPLLIFTSNATLGMELVNTRRK
EC_mntH	GFIRFHIPWLWVRRRT---MLPSFIVI-LMGLDPTRLVMSQVLLSFGIALALVPLLIFTSDSKLMDLIVNSKRVKQ
BS_ydaR	GFINRYIPLYVRRFIT---IIPPLIII-ASGVNPT-TALVLSQVVLISFGIAFALIPLIMFTSNKRIIMGSLINAKWIT
CAP0063	GFVNLSIPIINLRRIIT---MLPALIII-ALGINPM-RVVLVLSQVALSFILPFPIIQMILLIAGRKDLMGILVNNKKFTK
MT0951	GLLHWHSVPMVLVRRLIT---LGPALAIL-TLGFDP-TRTLVLSQLVLSFGIPFAVLPLVKLTGSPAVMGGDTNHRA
all17601	GFLQFRLPSWLRRLAIIPALITIILFGENSTSSLIVLSQLPFAVPLVMFTSNRRLMGFVNPLWLK
mlr2501	GFLRLRIPNWARRLLTRGLAIVPVVVVTALYGEKGTQGOLLVFSQVILSMQLPFAVVPLVQFVSDKKMGNLAI
DR1709	VIGWVIAVIIIAILNGLYLLWELL
STM2408	QVGWIIIVVLLVVAALNIWLLVGT
EC_mntH	TGWVIVVLLVVAALNIWLLVGT
BS_ydaR	VVSWLIAVVLVNAVLNVLIVDTF
CAP0063	IVGFIITATMIIILNIIILYLT
MT0951	WVGWVVAVMVSSLNVMLIYLTV
all17601	SLAWLVAIVIVGLNAWLLLQLS
mlr2501	ALAWVVAIAILVLFNKLLYDTL

S6A, Legend. Alignment for Nramp family of Mn-transporters. Membrane segments predicted by TMpred program (*S41*) are marked by blue, two motifs marked by yellow are necessary for activity (*S42*). DR1709 –*Deinococcus radiodurans*; STM2408 - *Salmonella typhimurium*; EC_mntA – *Escherichia coli*; BS_ydaR – *Bacillus subtilis*; CAP0063 – *Clostridium acetobutylicum*; MT0951 – *Mycobacterium tuberculosis*; all17601 – *Nostoc* sp.; mlr2501 - *Mesorhizobium loti*.

Daly_Fig. S6 (B) Alignment for periplasmic binding protein (DR2523).

DR2523	ALLAAACVGSGAAAAPLPVSATSSLADFVRQVGGSRVNVNIVPAGADAHTFQPSTGVIRSLVGSKVLFANGACLE
BS_mntA	ALTGCGTDSAGKSADQQLQVTATTSQIADAAENIGGKHVKVTLGKPGVDPHLYKASQGDTKLLMSADVVLVYSGHLLE
sll1598	EEVTAVTTEVQGETEKKVLTFTVLAQMVQNAGDKLVVSEITRIGAEIHYEPTPSDIVKAQDADLILYNGMNLLE
alr3576	GCASVDSRTPANADGKPQVVATSTIADLAQEVGEEIQLTGILKPGTDPHVYEPVPADSRVLEKAIDLILYNGYNLLE
Cg10027	AASLAFGITACSAVDTPDIVVTTNILGDVVSIVHGDSADVQVLMKPNADPHSGVUSAQDAAMEHAIDLIVANGLGLE
HI0362	IMTALALGLFAMQANAKFKVVTTFVIQDIAQNAGNAATVESITKPGAEIHEYEPKDVKQAQSADLIWNGLNLE
SPy0453	LLVACSSSTGKTAKSDFKLKVVATNSIADMTKAIAGDKIDLHSIVPIGQDPHEYEPLPEDVEKTSNADVIFYNGINLE
STM2861	AGIVAILALSPAYAKEKFKVITTFVIADMAKNVAGDAEVSSITKPGAEIHEYQPTPGDIKRAQGAQLILANGLNLE
SM02509	AMAALSLMPAAARAEEKLKAVTTFTVIADMAQNAGDAAVVSEITKPGAEIHNQOPTPRDLKAHDADLILWNGLNLE
TP0163	TGFTHAFGSKDAAADGKPLVVTIGMIADAVKNAQGDVHLKGLMGPVDPHLYTATAGDVWELGNADLILYNGLGLE
SP1650	ILVACASGKDDTSGQKLKVVATNSIADITKNIAGDKIDLHSIVPIGQDPHEYEPLPEDVKKTSEADLIFYNGINLE
DR2523	P----WLPLRLAAA---PRPVKELTAG---LKLHAGEGGAPPDPHAWWDASLALGYVKNVQTALSA-ADPAQATYA
BS_mntA	G----KMEDVLQKIG--EQKQSAVAEAIPKNKLIPAGEGKTFDPHWFWSIPLWIYAVDEIEAQFSK-AMPQHADAFR
sll1598	R----WFEQFLGNV--KDVPVSLLTEGIEPIADGPyTDKPNPHWMSPRNALVYVENIRQAFVE-LDPDNAKYYN
alr3576	P----GLIKLMLNAAG--SKARLAVGEVVKPLQLDKGKGEVVPDPHVGWSAENAVMNAIRDALIE-LSPKDREKYT
Cg10027	E----GLQSNVNDNAK-SQGVPVLEVGEHIDVIDYTES---PGVPDPHFWTDPARMTAATEVIAEELIKELDPSLTERIT
HI0362	R----WFERFFQNV--KDKPAVVTEGIQPLSIYEGPYKDAPNPWAWMSPSNALIYIENIKNALVK-YDPQNAAVYE
SPy0453	DGGQAWPTKLVKNAQTKNKDYFAVSDGIDVYLEGASEKCKEDPHAWLNLENGIIYSKNIAKQLIA-KDPKNKETYE
STM2861	R----WFERFYQHL--SGVPEVVSTGVKPMGITEGPYNGKPNPHWMSPAALIYVDNIRDALVK-YDPDNAQIYK
SM02509	L----WFERFFQNF--DGVPGVVVSEGVEPMGIAEGPTGKPNPHWMSPAALIYVDNIRDAFVQ-HDPNNAEVYK
TP0163	T----KMGEVFSKLRGSRLV--VAVSETI-PVSQRLSLEFAEFDPPHWFVDVKLWLSYSVKAVYESLCK-LLPGKTREFT
SP1650	TGGNAWFTKLVENEAKKTEKDYFAVSDGVDVYILEGQNEKGKEDPHAWLNLENGIIIFAKNIAKQLSA-KDPNNKEFYE
DR2523	KNAAYASAQIRAADAWAKKQFATLPPASQRKVVTHDLSGYFARRYGLTVIGAVIPGLSTEREPSARELATLASAVKKS
BS_mntA	KNAKEYKEDLQYLDKWSRKEIAHYPEKSRVLVTAIDAFAYFGNEYGFVKVG--LQLGSTSDYGLRDVQELVDLLTEK
sll1598	ANAAVYSEQLKAIQRQLGADLEQVPAQRFLVSCGAFSYLARDYGMEEI-Y-MWPINAEQQFTPQVOTVIEEVKTN
alr3576	QRASQLTDELQKLHWSINQQIQTIPPDKRKLITTHDAFQYYGRAYGLAIAGT-LIGISTEEQPSAQTVKRLVDSIKNI
Cg10027	QSAQHYREELVALDEEVTELLSGVAPENRKLVTNINVFGYLASRFNYTVIDTIIPGGSTILAAPSASDLNDISTAEDN
HI0362	KNAADYAQKIKQDLEPLRAKLAQIPEAQRWLVTSEGAFSYLAKDYNLKEG-Y-LWPINAEQOGTPQQVRKVIDLVRKN
SPy0453	KNLKAVYAKLEKLDEAKSKFDIAENKKLIVTSEGCFKYSKAYGVPSCA-Y-IWEINTEEGTPDQISSLIEKLKVI
STM2861	QNAERYKAKIRQMADPLRAELEKIPADQRWLVTSEGAFSYLARDNDMKEL-Y-LWPINADQGTPKQVRKVIDTIKKH
SM02509	ANAEAYKKKIEAAVAPIRTELERIPAERRWLVSSEGAFSYLARDFGMKEL-Y-LWPINADQGTPQQVRKVIDVVRAN
TP0163	QRYQAYQQQLDKLDAYVRRKAQSLPAERRVLVTAIDAFGYFSRAYGFEVKG--LGQVSTASEASAHDMQELAFAIQR
SP1650	KNLKEYTDKLDKESDKFNKIPAEKKLIVTSEGAFKYFSKAYGVPSCA-Y-IWEINTEEGTPEQIKTLVEKLRO
DR2523	GAKVILTENTVSTRALAQT---ASETG---ARIA-PPLYTALGPQAAAPA---KLTSKPSNTTWRRW
BS_mntA	QIKAVFVESSVSEKSIINAVVEGAKEKGHVTIG-COLYSDAMGEKGTKEGTYEGMFRHNINTITKAL
sll1598	NVPTIFCESTVSDKGQKQV--AQATG--ARFG-GNLYVDSLSTEEGPVPTFLDLEYDARVITNGL
alr3576	GVPAIFAETTINTPLIKTV---AEEAG--IKLAPNQLYSISGAKGSNGDSYIKMEANTRTIVEAL
Cg10027	NVPAIFTDSSPQRLAEVL---ASNAG--IDVQVVSIFTESLTDADGEAPTYISMQKINAERIASTL
HI0362	NIPVVFSESTISAKPAQQV--AKESG--AKYG-GVLYVDSLAKNGPVPTYIDLLNVTSTIVKGF
SPy0453	KPSALFVESSVDRPMETV---SKDSC--IPIY-SEIFTPSIAKKGKPGDSSYYAMMKWNLDKISEGL
STM2861	HIPAIFSESTVSDKPARQV--ARESG--AHYG-GVLYVDSLSEAADGPVPTYIDLLRVTSETIAKGL
SM02509	RIPVVFSESTISPDPAEQV--ARETG--AKYG-GVLYVDSLSEAADGPVPTYIDLLRVTSETIAKGL
TP0163	KLPAIFIESSIPHKNVEALRDAVQARGHVVQIG-GELFSIAMDAGTSEGTYYGMVTHNIDTIVAAL
SP1650	KVPSLFVESSVDRPMKTV---SQDTN--IPIY-AQIFTPSIAEQKGKEGDSYYSMKYNLDKIAEGL

S6B, Legend. Alignment for periplasmic binding proteins. Metal-binding site (marked by green) have been predicted on the basis of *TroA* (*Tro163*) and *PsaA* (*SP1650*) crystal structure (*S43-S45*) (marked by red). DR2523 -*Deinococcus radiodurans*; BS_ytdA -*Bacillus subtilis*; sll1598 - *Synechocystis sp.*; alr3576 – *Nostoc sp.*; Cg10027 - *Corynebacterium glutamicum*; HI0362 - *Haemophilus influenzae*; Spy0453 - *Streptococcus pyogenes*; STM2861 - *Salmonella typhimurium*; SM02509 – *Sinorhizobium meliloti*; TP0163 - *Treponema pallidum*; SP1650 - *Streptococcus pneumoniae*.

Daly_Fig. S6 (C) Alignment for ATPase (DR2284).

DR2284	LGVEGLTVRYGS-QVALENATLRFEAGQ FTAVI GPNAGAKS	TLLRALAGLLTDYEGRVTFD--PGHS--PRTCLSYVP
BS_mntB	VELDNVTVAYH-KKPVLQDISLQVPEGK LIGI GPNAGAKS	TLIKTILGLVPRASGDISIYGKDYKD--QRTRIGYVP
sll1599	ISVDPGVSVTYNNARALALYNATCTVEPGT ITALV GPNAGAKS	TLFKSIMGFLQPSQGRVRIGGFSVQKAQKQLMAYVP
all3575	INISHLGVHYR-TQEALRDVN CIVKPGRT IT ALVGPNAGAKS	TLMKAMIGLVPVSSGRVLYQNPKPLMQ--QLGKVAYVP
Cgl0029	LSVRNLCTCTYGN-HIALNNITARFPTGK ITALI GNSNGSGK	STLLETLAGMLAPRSGSIN-----N--LVPEIAFVP
HI0361	IWVNNDVTVRYNNNGHTAIHNMTFSLNST ICALV GPNAGAKS	STLFKSIMGLVKPQOGEIKLCCLPISQALKRNLVAYVP
SPy0454	ITTNNLCLVTDGNSNALEINVTEGPs IVGI GPNAGAKS	TFMKAILNLIDY-QGHVTVDGKDGRK--LGH TVAYVE
STM2862	ITVDQVTVTYRNGHTALRDAFQVPGGS IAALV GPNAGAKS	STLFKALMGFVHLAQGDITILQQSVNKALKKNLIAYVP
SM02508	IRVSGATVTYRNGH ICAL RDASFEIPTGT IAALV GPNAGAKS	STLFKAIMGFVRLAKGDISILGLTVPQALRNLVAYVP
TP0164	VQVDDLTLAYR-QKPVLWDVDVRIPEGV IEAI I GPNAGAKS	STLLKAIMGLLPLASGEVRFVGRPSK--ERRRVAYVP
SP1648	MIRIENLNSVSYKETLALKDISLVLHGPT LTG I GPNAGAKS	STLLKGMLGIIPH-QGQAFLDDKEVKKSL--HRIAYVE
DR2284	QQQTLDWGFPTVWDTAMMGRTGRLGWLWRPGPRDRQIVEDALRETGVYDLRSRHIGALSGGQRQRVLLARMLARQGH	
BS_mntB	QRGSVSDWFPTSPLDVVLMLGRYGRIGLLKRPKADVEMAKAALT KVGMDYAKRQISQLSGQQQQRVFLARALCQNA	
sll1599	QADEVDWNPVSVFDVVMGRYGYMNVLRI PAKRDKRLLVMESLERVMV KYDRQIGELSGGQKKRAFLARALA QEGK	
all3575	QRSQIDWTYPATVWDDVVMMGRVKTGWLRSFSAVRQVAKNALE RVMGMLDYCDRPICQQLSGGQQQRVFLARALA QQAD	
Cgl0029	QRSHVSHNLPITRQTVSMGRWSAKKNWQR LTAACDNIVDSCLDRLEISGLADRLGEVSGGQRQRALIAQGLAQOAP	
HI0361	QSEEVWDQFPVSVYDVVMGRYGYMNVLRI PAKIDKQKVQEAMQRVNIEHLAHRQIGELSGGQKKRVFLARALA QQSP	
SPy0454	QRSMIDYNFPIVKECVALGTYSKLGLFRRVGKKQFEQVDKVLKQVGLEDFGHRIKSLSGQFQRMVLVARCLIQESD	
STM2862	QSEEVWDWSFPVVLVEDVVMMGRYGHMGWLRRPTAHD HACVDAALARVDMQYEYRHRQIGELSGGQKKRVFLAR AIAQDGQ	
SM02508	QAEVWDWNPFPVVLVEDVVMMGRYGHMNMLRIPKKADHEAVET ALARVGMSEFRKRQIGELSGGQKKRVFLARALA QNGR	
TP0164	QRSAVWDWFPTTVFDVVLMSGSYGLCWILRPGKREKARARE AIEEVGMAGFLDRQISELSGGQQQRVFLARALVQDAD	
SP1648	QKINIDYNFPIKVKCEVCSLGLFPSIPLFRSLKAKHWKKVQE ALEIVGLADYAERQISQLSGGQFQRLVIARCLVQEAD	
DR2284	LLLLDE PLTGVDTVTTQEQLMALLRRQADQGRAVVMVTHD LEQARRWCDRIVLINRRVIADGTPD-AVYTPQNVEA	
BS_mntB	IYFMDEPFAGVDAATERAIMTLLAELKEKGKTVL VVHHDLQTAEDYFDWILLLHLRKIAFGPTE-NVFTIENLQKT	
sll1599	VILLEDEPFTGVDVTEKGMIDL MELRDEGHTLILISTHD LASISTFCDH TILLNRTILAQGKTE-ETFTKENLELT	
all3575	IFCFDEPLVGIDQK TQAVIFEV FH EAAANKIVL VVNHDLGEISHFDDLVLLNREL IATGSRQ-QVLTEDNLHQ A	
Cgl0029	LLLLDE PLAAVDASHASLIEDVINQQRNQGTTI LATHDLQAHQ-ADQIIALEKGII----KPQRKATESIKKR	
HI0361	IIILDEPFTGVDVKTENAIVD LQLREEGH LILVSTHNLGSVPDFCDQVVMINRTVIAAGKTE-DTFNQHNLEIV	
SPy0454	YIFLDEPFVGIDSVSEK IIIVD LKLKMAGKT I LIVHHDLSKVEHYFDKLMILNKH LVAYGNVC-EVFTVDTLSKA	
STM2862	VILLEDEPFTGVDVKT EARI DLLREL RDEGRTMLV STHNLGSVTEFC DYTVMIKGTVLASGPT E-TTFTAANLEQA	
SM02508	VILLEDEPFTGVDVKT EDAIIRLLVALREEGRVMLVSTHNLGSVPDFCDQVVMINRTVIAAGKTE-DTFNQHNLELA	
TP0164	LYFMDEPFQGVDAATEQAI VTLKLTKGRGKTLVVVHDLQTV AEYFDRVLLNVRVIAEGAVV-SAFTEEYVQRA	
SP1648	YILLDE PFAGIDSVSEEIIMNTLRLDKKAGKTVLIVHHDLSKIPHYFDQVLLVNREVIAFGPTK-ETFTETNLKEA	

S6C, Legend. Alignment for ATPases. Catalytically important motifs are marked by green. DR2284 -*Deinococcus radiodurans*; BS_ytDB -*Bacillus subtilis*; sll1599 - *Synechocystis sp.*; all3575 – *Nostoc sp.*; Cgl0029 - *Corynebacterium glutamicum*; HI0361 - *Haemophilus influenzae*; Spy0454 - *Streptococcus pyogenes*; STM2862 - *Salmonella typhimurium*; SM02508 – *Sinorhizobium meliloti*; TP0164 - *Treponema pallidum*; SP1650 - *Streptococcus pneumoniae*.

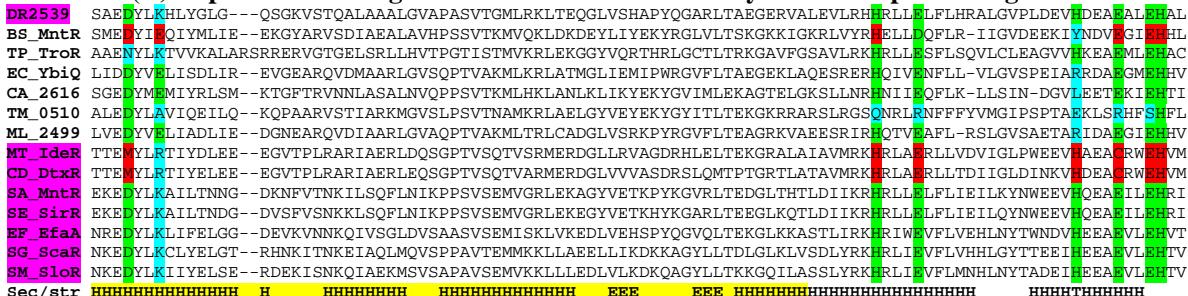
Daly_Fig. S6 (D) Alignment for permease (DR2283).

DR2283	FFVRALLAVSLVSILCALIGAWVVLRGLSYIGDAMSHAVLPGIVSAFLLKGNL-----LLGAAIAAVLTALGIGWIGRR
BS_ytgC	NTOWVLAGTLLLGTAGVLGSFVLLRKQSLIGDAMAHASLPGCLAFPLTGQKSLPFFLGLGAALAGLLGTCIQLIPRL
BS_ytgD	FEAWIATGVLVGVSCLGLTFLVLRSMAMLAIDAISHTVLLGIVGAFLVTGSLDGIYMFIGAAATGLLTAFLVQLLHSK
all3574	FMRORSLVIAILVGLICAVVGSYLMVQRALLGDAISHSVLPGLAIAFMVGANI----EVGAFIAGVLSITIAIWIRTR
s111600	FLIRAIWVSAFVGLCAVLSCYITLKGWSLMDGAISHAVPGVVLAYALNIPF----AIGAFTFGFATVAIGYVKSK
Cg10028	FISRALVAGCLAAILCSLIGTWTILRRLTFFGDAMSHGLPGVATASLGGNL----MFGAAISALIMSAVVWTSRK
HI0359	FMQNALLTALIVSIICALLSCYLVLKGWSLMDGAISHAVLPGVIVLAYLAGIPL----AIGAFFSGIFCSSLGVGYLKEN
HI0360	FMQNALLTALIVSIICALLSCYLVLKGWSLMDGAISHAVLPGVIVLAYLAGIPL----AIGAFFAGILAALSILWIJKS
SPr0456	FLQNLALITAVVIGIVGSAVGCFVILRSMSLMDGAISHAVLPGVVALSILGVNF----FIGAIIFGLLASVILITYIKEN
STM2863	YMLNAMWVSAVGGLCACFLSCYLMKGWSLIGDALSHSIVPGVAGAWMLGLPF----SLGAFLSSGLAAGSMSFLNQR
STM2864	FMVNALMVSIVIAIPCALLSVFLVLCWALMGDAMSHAVFPGVVLAYIVGIPL----AIGAFIAGLFCIATGYLDDN
SM02506	FMRNALLISVLVIAPTMLSCFLVLCWALMGDAMSHAVFPGVVVSVLYVGLPL----AVGAFTAGMCALLTGYLKEN
SM02507	YMNAMWVSAVGLGAVCAFLSAYLMLKGWSLIGDALSHSIVPGVAGAMGLPF----SLGAGFSGALAAGAMFLNQR
TP0165	TLCNVLVLTFLGLGSSGLVGSFAVLRRQSLPGDAVSHATLPGIVIAFLLTGTKSTEILLGAALSGLVTGTVVMLVMR
TP0166	TMTEVVLIAAVVSVSACALCGVFLVLRISLMSDAISHSVILGIVLGYFLSRTLSSFPVVGAVIAGICSVICABELQKT
DR2283	SGLQDS AIGIVFVGFMALGIVLLSRAPT---FTSDLSNFLIGNPLGV-----TPAD LWGALAVTLGVGGI
BS_ytgC	SKTKEDEAIGIVLVSFLVFFGVGILLLTIIQQQGAGGSQGLDSFLFGQAASL-----VRQD ILLIAGISAVLLLL
BS_ytgD	G-VQSDAIGVVFSTSFLAIGIVSVGA---NVHLDIEHSLMGEIAFPWNTVTFGVDIGPKAFWMLASVLVLNVVL
all3574	SPIKEADAAMGIVFSASFALGIGTITLTVVQK---DNKIDLNHFLFGNILGV-----TVDEVRDTAIIIAIVLTV
s111600	TRLKEDAVIGIVFTGFMLGLVLTKIPS---NVDLPHILFGPNVLGI-----SQD IIQTTLAGSITLIV
Cg10028	SSLSQDVSGISLQFITMLVSHGS---HAVDLTSFLFGDILGV-----RPSD IFIIIAIATVLLGLT
HI0359	SRLIKEDEATMIVGIVFSGMAIGLVMFTKQI---EEHLHILFGNVNLGV-----SHQE LIQSAVISAIIFCL
HI0360	SKLKEDAVIGIFLSTFFALGLLIVSLNPT---AVNVQNIILGNILGI-----ADED IYQVAAIIIGVCLVL
SPr0456	SVIKGDIATIGITFSSFLALGVILIIVAN---SSTDLFPHILFGNILAV-----QDSDKWITIGVSIIFVLLV
STM2863	SRLKEDAAIIGLIFSSFFGVGLFMVSLNPM---SVNIIQTIILGNVLA-----APAD IAQLAIIIGAVSLTI
STM2864	SRIKRDTVMGIVFSGMFAGLVLVYVIQS---EVHLDHILFGDMLGV-----SLGD IVQTSVIALGIALI
SM02506	SRIKRDTVMGVVFSGMFGLGLVLYTKQS---DVHLDHILFGDMLGI-----GWGD ILETGLLIALFAAGI
SM02507	TRLKEDTIIGLIFTSFGLGFLFMVSLSP---SVNIQTIVLGNILAI-----TPAD TLQLAIIIGVVSLSMI
TP0165	TKIDTCAQQGIVLGVFLGPFGFLLTHVOKSPQAAKAGLNKFILGQAAATI-----IQRD VLLIIAAEVVGILL
TP0166	GMVKSDA AVGLVFPAMFGLGVILVSLYAG---NVHLDTDAVLLGEI-GLAPLDRVSFFA SLPRS LVQMGSVLCGLLLL
DR2283	LTA IQKELLASFDPTEAR TVGLPVTRLNN LLLV LIGL VVVL TQV LGTTLSV SLLITSSAA RLLS---SLRT MLLL
BS_ytgC	CIV FKEFTLITFDL AFAK GLGIP VRF RLNG LLA CLIV CAVV IGL QTV GVL MA LIT PAIT ARY WTE---RLTG MIII
BS_ytgD	BS_ytgD
all3574	BS_ytgD
s111600	BS_ytgD
Cg10028	BS_ytgD
HI0359	BS_ytgD
HI0360	BS_ytgD
SPr0456	BS_ytgD
STM2863	BS_ytgD
STM2864	BS_ytgD
SM02506	BS_ytgD
SM02507	BS_ytgD
TP0165	BS_ytgD
TP0166	BS_ytgD
DR2283	AAALGILGGVGSLYASYYLDT -APGATIVL VNTAIFLL ALA FRRK
BS_ytgC	AGITGGVVG VAGTLL STMKG MATGPLMILS ATLL FLSM ICAPK
BS_ytgD	SALIGGLLSA VMG YFFAT WLNV -SISGAMA AMT GVCY AS AFL SPKA
all3574	GAGIGVFSSISGMYL SYFYNL -PSGPAIVL VV SGL FLL ALL FSPK
s111600	SVVSSV LSCVL GTL SYHF DV -STGGMIVV IL TTL FV IAMIGAPK
Cg10028	ASL LGCAE IY LG LI S WHAST -AAGATITL SAA IF FT ALL T KSA
HI0359	AIASSIA SLLIGV ILSY HF DA -STGACI ILL QAA FFVIA LAY SKI
HI0360	AIILGA VT SVGVY IS YY LDG -ATGGVIVL TLQ TLL FLV A FSPK
SPr0456	SSLLGALAS VL GLY LGY TF NV -AAG SSI VLT SAMM F L IS FV FSPK
STM2863	AVVIGSLTSFLG AWL SYWL DG -ATGGI I VV M QT LL FIT A F I F A PK
STM2864	AVS LS VITA FAG VY LS FV L DS -AP APTIV VV FA I FIA F I Y ATW
SM02506	AVMVAVAAS FCG VY LS F FIDS -AP APTIV VLL M T AMF L L A FAY STW
SM02507	SIAGA T S FV G VY AS Y FLD G -ATGGI I I V L Q T A I F L F A F V I A P K
TP0165	AAL FGG VSG VSG SV VSA QV P RL ST GP VIV L V LT G I A L V S I M L GP Q
TP0166	ASLLASCASISGLFLAVKIDG -SIAGA MAT MAG V L F AL V Y L F S P K

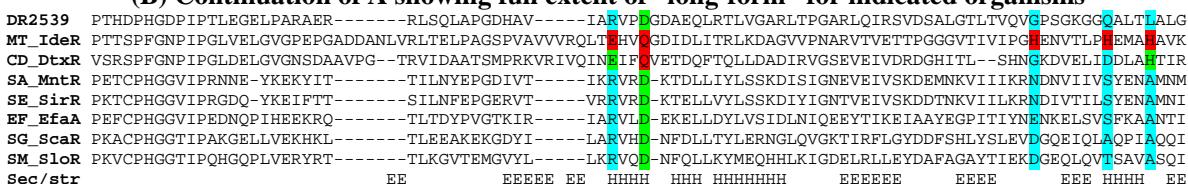
S6D, Legend. Alignment for permeases. Membrane segments predicted by TMpred program are marked by blue. DR2283 – *Deinococcus radiodurans*; BS_ytgC, ytg_D – *Bacillus subtilis*; slr2045 - *Synechocystis sp.*; all3574 – *Nostoc sp.*; Cgl0028 - *Corynebacterium glutamicum*; HI0359, HI0360 - *Haemophilus influenzae*; Spy0456 - *Streptococcus pyogenes*; STM2863, STM2864 - *Salmonella typhimurium*; SM02506, SM02507 – *Sinorhizobium meliloti*; TP0165, TP0166 - *Treponema pallidum*.

Daly_Fig. S7

(A) Representative alignments for MntR/DtxR family of transcriptional regulators



(B) Continuation of A showing full extent of “long-form” for indicated organisms



S7, Legend. (A) “short form” alignments. (B) Species that encode the “long form” alignments are marked in magenta (panel A), showing additional C-terminal SH3-like fold domains in panel B (*feoA*-like). DR – *Deinococcus radiodurans*, BS – *Bacillus subtilis*, TP – *Treponema pallidum*, EC – *Escherichia coli*, CA – *Clostridium acetobutylicum*, TM – *Thermatoga maritima*, ML – *Meso loti*, MT – *Mycobacterium tuberculosis*, CD – *Corynebacterium diphtheriae*, SA – *Staphylococcus aureus*, SE – *Staphylococcus epidermidis*, EF – *Enterococcus faecalis*, SG – *Streptococcus gordonii*, SM – *Streptococcus mutans*. For MT_IdeR, CD_DtxR and BS_MntR, the crystal structures were solved: residues marked by red were shown to be important for metal-binding. The corresponding residues for other proteins are marked by green (if they are the same) and blue (if they are different). The DNA binding domain is marked by yellow.

Supporting Online Tables S1 to S3

Table S1. DNA repair, replication and recombination related genes that are absent in *D. radiodurans* but present in *E. coli* (EC), in comparison with *S. oneidensis* (SO), *P. putida* (PP), *L. plantarum* (LP), *E. faecium* (EF), *Rubrobacter xylanophylus* (RX), *Kineococcus radiotolerans* (KR), and *D. geothermalis* (DG).

Pathway ^A	Protein description and comments ^B	SO ^C (S14)	PP (S47)	EC ^D (COG)	LP (S4)	EF ^E (OR NL)	RX (S4)	KR (S4)	DG (S4)	DR (S4)	COG number
BER	3-methyladenine DNA glycosylase I	SO0016 SO4696	PP0062	Tag	lp_0296	+	-	+	-	-	COG2818
VSP	strand-specific, site specific, GT mismatch endonuclease; fixes deamination resulting from Dcm	-	-	Vsr	-	-	-	-	-	-	COG3727 (most gamma do not have it)
RER	Endonuclease /Holliday junction resolvase	-	-	RusA/ YbcP	lp_0647	-	-	-	-	-	COG4570
RER	Helicase/exonuclease	SO2148	PP4673	RecB	lp_2693	+	-	+	-	-	COG1074
RER	Helicase/exonuclease	SO2149	PP4674	RecC	-	-	-	+	-	-	COG1330
DR	O6-methyl-guanine-DNA methyltransferase; transcription activator/repressor	SO3126	PP0706	Ada	lp_2848	- + +	- + +	- + -	- - -	- - -	COG2162 COG0350
DR, BER(?)	Unknown	SO1098	PP3400	AlkB	-	-	-	-	-	-	COG3145
DR	DUTPase	SO4250	PP5286	Dut	lp_0604	+	+	+	-	-	COG0756
DR	dCTP deaminase	SO2616	PP1100	Dcd	-	-	-	+	+	-	COG0717
BER	Endonuclease IV	-	-	Nfo	lp_1976	-	-	-	-	-	COG0648, some gamma do not have it
DR	Photolyase	SO3384	PP0739	PhrB	-	-	+	+	-	-	COG0415
mMM	Endonuclease	SO1330	-	MutH	-	-	-	-	-	-	COG3066
mMM	GATC-specific N-6 adenine methyltransferase; imparts strand specificity to mismatch repair.	SO0289	-	Dam	lp_2454	-	+	-	-	-	COG0338
SOS	DNA polymerase II	SO1820	PP2393	PolB	-	-	-	-	-	-	COG0417
mMM, RER	Exodeoxyribonuclease I	SO2790	PP1365	SbcB	-	-	-	-	-	-	COG2925
mMM	site-specific C-5 cytosine methyltransferase; VSP is targeted toward hot-spots created by dcm	-	-	Dcm	-	+	+	+	-	-	COG0270, some gamma do not have it
MM, RER	Specific function unknown (predicted nucleotidyltransferase)	SO1114	PP1203	DinP	lp_2280	+	-	+	-	-	COG0389
RER	exonuclease VIII	-	-	RecE	-	-	-	-	-	-	No COG, most

											gamma do not have it
RER	annealing protein	-	-	RecT	lp_0641	-	-	-	-	-	COG3723 most gamma do not have it
SOS	predicted helicase; SOS inducer	SO1819	PP1125	DinG	lp_0308	+	-	+	-	-	COG1199
SOS	Error-prone DNA polymerase; in conjunction with umuD and recA, catalyzes translesion DNA synthesis	SOA001 ₂	-	UmuC	-	+	-	-	-	-	COG0389
SOS	In conjunction with umuC and recA, facilitates translesion DNA synthesis; autoprotease	SOA001 ₃	-	UmuD	-	-	-	-	-	-	COG1974
BER	Predicted acyltransferase; predicted DNA-binding protein	SO4248	PP5284	RadC	lp_2320	+	+	-	+	-	COG2003

Footnotes for Table S1:

^ABased largely on Aravind *et al.* (1999) (S46), with modifications.

^BAbbreviations of DNA repair pathways: DR- direct damage reversal; BER – base excision repair; NER – nucleotide excision repair; mMM – methylation-dependent mismatch repair; MMY – MutY - dependent mismatch repair; VSP – very short patch mismatch repair; RER – recombinational repair, SOS – SOS repair; MP – multiple pathways; putative, unconfirmed repair pathways are designated by a question mark.

^CThe gene names are from *E. coli*, whenever an *E. coli* ortholog exists, or from *B. subtilis* (with the prefix BS_).

^DCOG database.

^EORNL, draft annotation at Oak Ridge National Laboratory, Oak Ridge TN, USA.

Table S2. *D. radiodurans* genes coding for replication, repair and recombination related functions in comparison with *S. oneidensis* (SO), *P. putida* (PP), *E. coli* (EC), *L. plantarum* (LP), *E. faecium* (EF), *R. xylanophylus* (RX), *K. radiotolerans* (KR), and *D. geothermalis* (DG) (S4).

Pathway ^A	Protein description and comments ^B	SO ^C	PP	EC ^D	LP	EF ^E	RX	KR	DG	DR	COG and Commet (BLAST result)
mMM?	Adenine-specific DNA methylase	-	-	YhdJ	-	-	+	-	-	DRC0020	COG0863, some gamma do not have this gene
DR	O-6-methylguanine DNA methyltransferase	SO3126, SO2532	PP3017, PP1356	Ogt, YbaZ	lp_2848 -	+ -	+ -	+ +	- +	DR0428	COG0350, COG3695
DR	8-oxo-dGTPase. D.r. encodes additional 22 paralogs; only some predicted to function in repair	SO0410	PP1348	MutT	lp_0119	+	+	+	+	DR0261	COG0494
DR, BER	3-methyladenine DNA glycosylase II; DR2584 is of eukaryotic type	SO3127	PP0705	AlkA	- lp_1991	- + +	+ + +	+ + +	+ + +	DR2584 DR2074	COG0122 COG2094
BER, MMY	8-oxoguanine DNA glycosylase & AP-lyase, A-G mismatch DNA glycosylase	SO3368	PP0286	MutY	lp_3349	+	-	+	+	DR2285	COG1194
BER	Endonuclease III & thymine glycol DNA glycosylase; DR0928 and DR2438 are of archaeal type and DR0289 is close to yeast protein	SO2514	PP1092	Nth	lp_2860	+	+	+	+	DR2438 DR0289 DR0928	COG0177
BER	Formamidopyrimidine & 8-oxoguanine DNA glycosylase	SO4726	PP5125	MutM/ Fpg	lp_1509	+	+	+	+	DR0493	COG0266
BER	Endonuclease V	-		Nfi/ YjaF	-	-	+	-	-	DR2162	COG1515, some gamma do not have this gene
BER	DNA polymerase I	SO4669	PP0123	PolA	- lp_1508	- +	+	+	+	DR1707	COG0258+ COG0749
BER	Uracil DNA glycosylase; DR0689 is a likely horizontal transfer from a eukaryote or a eukaryotic virus	SO3654	PP1413	Ung	lp_0806 -	+	-	+	?	DR0689 DR1663	COG0692, No COG
BER	G/T mismatch-specific thymine	-	-	Mug/ ygfF	-	-	-	-	+	DR0715	COG3663 many

	DNA glycosylase, distantly related to DR1751; Present as a domain of many multidomain proteins in many eukaryotes										gamma do not have this gene
BER	Uracil DNA glycosylase	-	-	-	lp_0048	+	+	+	+	DR1751	COG1573
BER	Exodeoxyribonuclease III	SO3037	PP2890	XthA	lp_0812	+	-	+	+	DR0354	COG0708
NER, BER	Predicted ATP-dependent protease	SO1226	PP4644	Sms/RadA	lp_0606	+	+	+	+	DR1105	COG1066
NER	transcription repair coupling factor; helicase	SO2255	PP2148	Mfd	lp_0539	+	+	+	+	DR1532	COG1197
NER	ATPase, DNA binding	SO4030	PP0483	UvrA	lp_0773	+	+	+	+	DR1771, DRA0188	COG0178
NER	Helicase	SO2506	PP1974	UvrB	lp_0772	+	+	+	+	DR2275	COG0556
NER	Nuclease	SO1861	PP4098	UvrC	lp_2109	+	+	+	+	DR1354	COG0322
NER, mMM, SOS	helicase II; initiates unwinding from a nick; DR1572 has a frameshift	SO0467	PP5352	UvrD	lp_1144 lp_0432	+	+	+	+	DR1775, DR1572	COG0210, COG3973
mMM, VSP	predicted ATPase	SO0601	PP4896	MutL	lp_2297	+	-	-	+	DR1696	COG0323
mMM, VSP	ATPase; DR1039 has a frameshift	SO3431	PP1626	MutS	lp_2271 lp_2298	+	+	-	+	DR1976, DR1039	COG1193 COG0249
MM	Exonuclease VII, large subunit	SO3294	PP1027	XseA/nec7	lp_1560	+	-	+	+	DR0186	COG1570
MM	Exonuclease VII, small subunit	SO1527	PP0529	XseB	lp_1601	+	-	+	+	DR2586	COG1722
RER	Exonuclease subunit, Predicted ATPase	SO2843	PP2024	SbcC	lp_1548	+	+	+	+	DR1922	COG0419
RER	Exonuclease	SO2844	PP2025	SbcD	lp_1416	+	+	+	+	DR1921	COG0420
RER, SOS	Recombinase; ssDNA-dependent ATPase, activator of LexA autoproteolysis	SO3430	PP1629	RecA	lp_2301	+	+	+	+	DR2340	COG0468
RER	Helicase/exonuclease; Contains three additional N-terminal helix-hairpin-helix DNA-binding modules; closely related to RecD from <i>B.subtilis</i> and Chlamydia	SO2147	PP4672	RecD	lp_2168	+	-	+	+	DR1902	COG0507
RER	Predicted ATPase; required for daughter-strand gap repair	SO0010	PP0012	RecF	lp_0005	+	+	+	+	DR1089	COG1195
RER	Holliday junction-specific DNA helicase; branch migration inducer	SO4364	PP5310	RecG	lp_1627	+	+	+	+	DR1916	COG1200
RER	Single-stranded DNA-specific	SO0952	PP1477	RecJ	lp_2087	-	+	-	+	DR1126	COG0608

	exonuclease										
RER	Predicted ATPase	SO3462	PP4729	RecN	lp_1605	+	+	+	+	DR1477	COG0497
RER	Required for daughter-strand gap repair	SO1350	PP1435	RecO	lp_1966	+	-	+	+	DR0819	COG1381
RER	Helicase; suppressor of illegitimate recombination	SO4241	PP4516	RecQ	lp_0962	+	-	+	+	DR1289	COG0514
RER	Required for daughter-strand gap repair	SO2015	PP4267	RecR	lp_0700	-	+	+	+	DR0198	COG0353
RER	Holliday-junction-binding subunit of the RuvABC resolvosome	SO2430	PP1216	RuvA	lp_2287	+	+	+	+	DR1274	COG0632
RER	Helicase subunit of the RuvABC resolvosome	SO2429	PP1217	RuvB	lp_2286	+	+	+	+	DR0596	COG2255
RER	Endonuclease subunit of the RuvABC resolvosome	SO2431	PP1215	RuvC	-	-	+	-	+	DR0440	COG0817
MP	Polymerase subunit of the DNA polymerase III holoenzyme	SO1644	PP1606	DnaE	lp_1899	+	+	+	+	DR0507	COG0587
MP	3'-5' exonuclease subunit of the DNA polymerase III holoenzyme	-	PP4141	DnaQ	lp_0811	+	-	+	+	DR0856	COG0847
MP	DNA ligase	SO2896(NAD)=lig A, SO2204(ATP),	PP4274	LigA yicF	lp_1145	+	+	+	+	DR2069	COG0272
MP	Single-strand binding protein; D. radiodurans R1 has three incomplete ORFs corresponding to different fragments of the SSB	SO4028	PP0485	Ssb	lp_0010	+	+	+	+	DR0099	COG0629
SOS	Transcriptional regulator, repressor of the SOS regulon, autoprotease	SO1644	PP2143, PP3116	LexA	lp_2063	-	+	+	-	DRA0344 DRA0074	COG1974 No COG
VSP?	Uncharacterized proteins related to vsr	-	-	YcjD	-	-	-	+	-	DR0221, DR2566	COG2852, No COG
?	Uncharacterized family of presumably metal-dependent enzymes	-	-	Bs_Din B	-	-	-	+	+	13 homologs	COG2318
DR	xantosine triphosphate pyrophosphatase, prevents 6-N-hydroxylaminopurin mutagenesis	SO3358	PP5100	HAM1/ YggV	lp_2267	-	-	+	+	DR0179	COG0127

NER	UV-endonuclease; Activity was characterized in Neurospora	-	-	Uve1/ BS_Ywj D	-	-	+	-	-	DR1819	COG4294
NER	DNA or RNA helicase of superfamily II; also predicted nuclease; Contains an additional McrA nuclease domain	SO2744	-	YejH/ rad25	-	-	+	+	-	DRA0131 _1_2	COG1061
?	Topoisomerase IB	-	PP3831	-	-	-	-	-	+	DR0690	COG3569
?	3'->5' nuclease; Related to baculoviral DNA polymerase exonuclease domain	-	-	-	-	-	-	-	-	DR1721	No COG
?	Ro RNA binding protein; Ribonucleoproteins complexed with several small RNA molecules. Involved in UV-resistance in Deinococcus	-	-	-	-	-	-	-	-	DR1262	No COG
?	Predicted nuclease and Zinc finger domain containing protein. An ortholog is present in Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	DR1757	No COG
?	MRR-like nuclease; Restrictase of the RecB archaeal Holliday junction resolvase superfamily	SO0304? -	PP3694 ?	- Mrr	lp_1858	-	-	+	-	DR1877 DR0508 DR0587	COG1787, COG1715 (most gamma do not have this gene)

Footnotes for Table S2 are as for Table S1.

Table S3. SOD/catalase/peroxidase systems that defend against oxidative stress.

Strain	SOD/Catalase/Peroxidase								source of counting
	sodA/B COG0605	sodC COG203 2	katE/A/B COG0753	Mn- catalase COG3546	katG COG0376	peroxidase COG2837	mauG COG18 58	btuE GOG0386	
<i>D. radiodurans</i> (whole sequence)	DR1279 (SodA)	DR1546, DRA020 2_1	DRA025,D R1998 and related DRA0146	-	-	DRA0145	DRA03 01	-	COG database
<i>D. geothermalis</i> (draft sequence)	DG2738 (SodA)	-	DG2896	-	-	-	-	-	Draft COG assignment by correlation with DR genome, and draft annotation at ORNL
<i>E. faecium</i> (draft sequence)	-	-	-	Efa2038	-	-	-	Efa225	Draft annotation at ORNL
<i>L. plantarum</i> WCFS1 (whole sequence)	-	-	lp_3578	-	-	lp_3430	-	lp_0220	Annotation at NCBI, CDD
<i>E. coli</i> (whole sequence)	sodA, sodB	sodC	katE	-	katG	ycdB, yfeX	yhjA	btuE	COG database
<i>P. putida</i> (whole sequence)	PP0946, PP0915	-	PP0115, PP0481	-	-	PP3248	PP2943	PP0777, PP1686, PP1874	Annotation at NCBI, CDD
<i>S. oneidensis</i> (whole sequence)	SO2881	-	SO1070	-	SO0725, SO4405	SO0740	SO2178	SO1563	Annotation at NCBI, CDD

Footnotes for Supplemental Table S3:

Analyzed using Cluster of Orthologous Group (COG) database:

COG0605 Superoxide dismutase

COG2032 Cu/Zn superoxide dismutase

COG0753 Catalase

COG3546 Mn-containing catalase

COG0376 Catalase (peroxidase I)

COG2837 Predicted Fe-dependent peroxidase

COG1858 MauG, Cytochrome c peroxidase; CCP_MauG, Di-haem cytochrome c peroxidase. This is a family of distinct cytochrome c peroxidases (CCPs) that contain two haem groups. Similar to other cytochrome c peroxidases, they reduce hydrogen peroxide to water using c-type haem as an oxidisable substrate. However, since they possess two, instead of one, haem prosthetic groups, bacterial CCPs reduce hydrogen peroxide without the need to generate semi-stable free radicals. The two haem groups have significantly different redox potentials. The high potential (+320 mV) haem feeds electrons from electron shuttle proteins to the low potential (-330 mV) haem, where peroxide is reduced (indeed, the low potential site is known as the peroxidatic site). The CCP protein itself is structured into two domains, each containing one c-type haem group, with a calcium-binding site at the domain interface. This family also includes MauG proteins, whose similarity to di-haem CCP was previously recognised.

COG0386 Glutathione peroxidase; reaction: 2 glutathione + H₂O₂ = glutathione disulfide + 2 H₂O.

Supporting Online References (S1-S50)

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